Date:	December 12, 2000
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To:	BLA # 99-2865; STN# 103979
Through/With:	Barry Cherney, Ph.D., Biologist, DTP Amy Rosenberg, M.D., Director, DTP
Product:	Fabrazyme: Recombinant human alpha-galactosidase beta
Proposed Use:	Enzyme replacement therapy for treatment of Fabry disease
Sponsor:	Genzyme Corp.

Rationale and Background:

Fabrazyme (recombinant α-galactosidase; agalsidase beta) is intended as a life-long enzyme replacement therapy for Fabry disease (an X-linked inborn error of metabolism characterized by subnormal or absent activity of endogenous lysosomal hydrolase, α-galactosidase). Recombinant human α-galactosidase is a homodimer of two 398 amino acid subunits.

Native human α-galactosidase is an enzyme of approximately 100 kD that catalyzes the specific removal of the terminal galactose from the GL-3 (globotriasylceramide). This step results in the production of ceramide dihexoside (globolactosylceramide; GL-2) and leads to the formation of ceramide (precursor for glycosphingolipids) in the pathway of sphingosine formation. Deficiency of α-galactosidase leads to progressive accumulation of the substrate, GL-3, predominantly in the lysosomes of endothelial, perithelial and smooth-muscle cells of blood vessels. GL-3 accumulation also occurs in ganglion cells of the autonomic nervous system, cardiomyocytes of the heart, epithelial cells of glomeruli and tubules in the kidney, epithelial cells of the cornea, and cell lines of many other tissues. Excessive accumulation of GL-3 in the vascular wall results in narrowing and thrombosis of arteries and arterioles. Clinical consequences of tissue GL-3 accumulation are characterized by progressive impairment of tissue and organ function. In the second or third decade of life, the onset of renal insufficiency typically presents and results in end-stage renal disease requiring dialysis and/or transplantation in the fourth and subsequent decades. Progressive disease burden in symptomatic individuals results in a substantially decreased life expectancy, as symptomatic Fabry disease is largely fatal. Fabry related deaths are typically due to renal failure, cardiac disease or cerebrovascular disease.

Since GL-3 accumulation in endothelial cells accounts for most of the clinical disease pathology due to a deficient α-galactosidase enzymatic activity, the approach is to attempt replacement of the deficient enzyme.

Table of Contents:

I. Characterization of the Cell Substrate for Fabrazyme production

A. Creation of the Vector

The active component is produced by growth of a recombinant Chinese Hamster Ovary (CHO) cell line transfected with a recombinant expression vector containing the cDNA coding region for human alpha-galactosidase (hαGAL) and purified from the cell culture media by chromatographic methods. A schematic of ----------, the vector expressing human alpha-galactosidase, is presented in Figure IIC-1.

The gene encoding human alpha-galactosidase (hαGAL) was isolated at Genzyme. --
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---.

Plasmid Map/Vector Construction

The parent vector for ---------- is ----------. This vector contains an ---------- transcriptional unit. The vector containing full-length hαGAL coding region, ----------, was used to create the hαGAL expression vector as outlined in Figure IIC-2.

The resulting vector, ----------, that was used to express hαGAL, contains the following elements:

1. ---
2. ---
3. ---
4. ---
5. ---
6. ---

B. Origin and History of the Parental Cell Line

The Chinese Hamster Ovary host cell line, CHO ---
---, was obtained originally from ---
---. CHO ---

---. This cell line was named ---
---. Characterization of the cell line in the Master Cell Bank (MCB) confirms that there have been no virus-like particles (other than retrovirus particles) carried over from the cell line. For further information on cell bank purity refer to Part IIC1.4 of the submission.

C. Creation of the Cell Substrate

1. Creation of the Production Cell Bank

--------- cells were transfected with vector ---------. ------- days after transfection, cells were reseeded at a ---------- dilution into flasks containing medium and ---------- ---------- Conditioned medium was removed and assayed for secreted alpha-galactosidase (αGAL) activity using the enzyme activity assay (----------). Cells were then ----------, counted, and passaged into the next higher level of ----------. Amplification was continued through ----------

Cells in which αGAL productivity was determined to be highest (----------) were cloned by limited dilution. Based on activity data, -- clonal lines were chosen and analyzed for stability of r-hαGAL productivity in the absence of -----. These studies were performed for greater than --- generations to ensure suitable stability for production scale-up. Based on the criterion of stable protein production in the absence of ------, clone ---------- was chosen as the candidate cell line for scale up and production optimization.

2. Characterization of the Master Cell Bank (MCB)

The MCB intended for use was prepared from one vial of the ---------- clonal cell line derived from the production cell bank. Cells were grown in ---------- containing ----------. Approximately ---------- cells per ----- ml were aliquoted per ampule. A total of ---- ampules, of the ---- Master Seed, ---------- MCB were frozen and stored in ----------. The rate of use ----------

The tests performed on the MCB are listed below. An explanation of the methods follows.

Test	**Method**	**Results**
Sterility	----------	----------
Viability	----------	----------
Mycoplasma	---------- ----------	----------
Retroviruses	---------	----------
	----	---------- ----------
----------	----------	----------
Adventitious agents, broad screen	----------	----------
	----------	----------
----------	----------	----------
----------murine retroviruses	----------	----------

----------murine retroviruses	----------	----------
Species identity	----------	----------
	---------- ----------	---------- ---------- ---------- ----------

Comments: Appropriate testing was done on the MCB as per ICH Q5A. This ensures safety and reproducibility. The MCB was of Chinese hamster origin and was free of adventitious agents. The endogenous retrovirus particles found are known to exist in CHO cells. Thus, the MCB is acceptable.

Test methods:

Sterility
Sterility testing of cell banks was performed at either ---------- ---------- ----------. ---------- ---------- ---------- ---------- ----------. The positive controls were not stated in Appendix IIV-6.

Viability
The MCB was tested for viability at Genzyme. The exact method was not detailed. The specification for sufficient viability was: -------%.

Mycoplasma
Mycoplasma testing of the MCB, WCB, and --- was performed at ---------- or ----------. A ---------- assay with ----- cells was used for non-cultivatable species. ------- ---------- ---------- were used for cultivatable species---------- ---------- were the positive controls.

Retroviruses: ----------
---------- was performed on a fixed cell pellet by ---------- for the MCB and ---- for the detection of contamination of the cell banks by viruses. ----- cells per pellet were examined for the presence of types A, B, C, D, and R particles. The percentages of each type of particle were noted, however the exact number should also be given.

Retroviruses: ----------
The detection of ---------- in the presence of ---------- was done on the MCB and ---- by ----------. Samples of MCB and ------ were analyzed for the presence of type B, C, and D retrovirus ----- activity and ----------activity. This method utilizes ----------. The presence or absence of ----- is

determined by measurement of --. The positive controls were ------
----
-------------------- were the negative controls.

------------------------------ -----------. --
---------------. ---

Adventitious Agents Broad Screen, In Vitro
This test was done on the MCB, WCB, ----- by both ---------------------. The test article (cell lysate) was ---
----

--.

Adventitious Agents Broad Screen, In Vivo
Testing was done on the MCB, WCB, ---- by --

--- --------------------- --- ------------

--- ----------

Detection of ---------- Murine Retroviruses
---------- assay was done on the MCB and ---- by ----------

Detection of ---------- Murine Retroviruses
---------- assay was done on the MCB and ----- by ----------

Species Identity Test Using ----------
---------- analysis and ---------- antisera testing (done on the MCB, WCB, ---- by ----------) were used to verify the hamster identity of the cell line. ---------
---

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3. Characterization of the Working Cell Bank (WCB)

A WCB was created from a single vial of the MCB. A total of ----- vials (approx----- ---- viable cells per vial) were prepared for the WCB, which were frozen and stored in ---- ----------. The current rate of use is about ----- ampules per year.

The tests performed and the results are listed below. Test methods were the same as those used above for the MCB.

Test	**Method**	**Results**
Sterility	----------	----------
Mycoplasma	---------- ----------	----------
----------	----------	----------
Adventitious agents, broad screen	----------	----------
	----------	----------

Species identity	----------	----------
	---------- ----------	---------- ---------- ---------- ---------

Comments: The WCB was appropriately tested to ensure safety and reproducibility. It is of Chinese hamster origin and is free of adventitious agents. Thus, it is acceptable.

In volume 2, section IIC, pages 107-110 the sponsor describes action to be taken should the need for production of a new MCB or WCB arise. ----------
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4. ---------- (----)

Test	**Method**	**Results (------30L)**
----------	---------- ----------	----------
----------	-----	----------
	---	---------- ----------
---------- ----------	----------	----------
	----------	----------
----------	----------	----------
---------- ---------- ----------	----------	----------
---------- ----------	----------	----------
----------	----------	----------
	---------- ------	---------- ---------- ---------- -------

Test	**Method**	**Results (-----340L)**
----------------------------	----------------------------------- -------	-------------
--------------------	----	---------------------------------
	---	----------------------------------- ------------
----------------------------------- -------------	-----------------	-------------
	-----------------	-------------
-----------------------	-----------------	-------------
----------------- ---------------- ----------	-----------------	-------------
------------------ --------------- ------------	-----------------	-------------
---------------------	----------------------	-------------------------------
	----------------------------------- ---------	----------------------------------- ----------------------------------- ----------------------------------- ----

Comments: ---.

D. Genetic Consistency of the Cell Substrate

1. 30L scale

--.

THESE PAGES DETERMINED NOT TO BE RELEASABLE

Comments: ----------, copy number, ------, and sequence were determined for two process sizes (30L, 340L) by ----------. Comparing the MCB, WCB and ------ there were no changes in ----------, copy number, sequence, or ----------. The MCB, WCB and ------ seem stable enough to provide a consistent product. Testing has been done such that if any changes in copy number, deletions, insertions, or changes in ----------, these would be detected.

E. Adventitious Agent Screening During Manufacture

1. Animal-derived Raw Materials (COAs are contained in Appendices IIV-1 to IIV-3)

   A) ---------- was obtained from BSE-free USDA-inspected herds in the United States and from BSE-free Ministry of Agriculture-inspected herds in New Zealand. The serum was tested as per 9CFR 113. ------- was used to create the MCB, WCB, and is used routinely in Fabrazyme production.
   B) ---------- was obtained from the United States. It was tested for ----------. Tests were negative. --------- was used to create the MCB and WCB. It is not used in production.
   C) ---------- was obtained from BSE-free herds in the United States and tested as per 9CFR 113. This was used to create the development cell bank, but not the MCB or WCB. It is not used in production.

Comments: The raw materials of animal origin were appropriately tested for viral contamination.

2. Drug Substance (-- process qualification lots)
   The bioreactors are ----------. Harvest material is collected for ---------- days before being transferred for testing. Summary of the characterization of the -- lots as compared with a reference standard is found in Table IIC-44.

In-Process Controls For Harvest Materials. From Table IIC- 47

Test	**Method**	**Specification**
----------	----------	----------
----------	----------	----------
---------- ----------	----------	----------
----------	----------	----------
----------	------	----------

Comments: The animal-derived raw materials and the harvest materials have been appropriately tested for adventitious agents. The in-process testing scheduled during manufacture should be sufficient to detect any potential contamination.

II. Viral Clearance Studies

Studies to measure both the robustness and clearance of retroviruses in particular were done using a scaled-down process along with the model viruses listed below. This is allowable. Both new and used column resins were tested.

The viral clearance studies, cytotoxicity/interference studies, and inactivation by cleaning solutions were done at both ----------. The columns are listed below in the order of use (----------). There is ---------- step in the Fabrazyme manufacturing process.

From Tables IIV-14 to IIV-22. Scaled-Down Chromatography Column Process Parameters:

Column	Diameter (cm)	Bed height (cm)	Bed Vol (ml)	Scale down ratio	Load FR (cm/hr)
----------	----	----	----	-------	----
----------	----	----	----	-------	----
----------	----	----	----	-------	----
----------	----	----	----	-------	----

*FR= flow rate;

From Tables IIV-14 to IIV-22. Manufacturing Chromatography Column Process Parameters:

Column	Bed height (cm)	Bed Vol (L)	Diameter (cm)	Load FR (cm/hr)
----------	----------	----------	---	-----
----------	----------	----------	---	-----
----------	----------	----------	---	-----
----------	----------	----------	---	-----

*FR= flow rate;

A. ----------

This model virus is a medium to large (----------), enveloped, ssRNA virus capable of infecting --------- cell lines. The reduction of -------- was monitored using infectivity assays on ---------- ------. The negative control was ----------, the positive control was ----------. The assay was done in ---------- and the results are listed in Appendix IIV-16, vol 23.

1. ---------- Column (----------)
   Load Buffer: ----------

Elution Buffer: ----------

	New resin	Used resin (--- cycles)
Input Viral Load (Log $_{10}$ PFU)	-----	-----
Output Viral Load (Log $_{10}$ PFU)	-----	-----
Log $_{10}$ Reduction	---------	----------

2. ---------- Column (----------)
   Load Buffer: ----------.

   Elution Buffer: ----------

	New resin	Used resin (--- cycles)
Input Viral Load (Log $_{10}$ PFU)	-----	-----
Output Viral Load (Log $_{10}$ PFU)	-----	-----
Log $_{10}$ Reduction	---------	--------

3. Cytotoxicity/Interference Assays

   --------- infectivity in -------- cells was used to assess cytotoxicity and interference. The negative control consisted of ----------, the positive control was ----------. No cytotoxicity was found in the samples of one study. In another study, the ---------- column buffers were diluted ----- to reduce toxicity. Viral interference was observed in undiluted samples of ---------- column buffers. These data were taken into account when evaluating the viral clearance studies.

Comments: The cumulative log 10 reduction of ---------- would be:

	New	Used
---------- Column	----------	----------
--------- Column	----------	----------
Total reduction:	----------	

The infectivity assays used appropriate methods and controls. The sponsor states that the clearance mechanism for enveloped viruses by the --- column could be either removal due to ----------.

In order to determine whether ----- log $_{10}$ is sufficient clearance, the ---------- particles per dose must be calculated. The sponsor did this (Vol. 3, pages 208-209, section IIV 3.3 and Vol. 22, Appendix IIV-15) and stated that there could be ---- Log $_{10}$ particles per dose. Given the clearance (----- Log $_{10}$), this means ------ Log $_{10}$ clearance remains, or one particle per ---- doses. ----------

----------This is further explainedin the Summary section 'E' below.

B. ----------

This dsDNA virus is large (----------), enveloped, and a member of the ---------- ---------- family. The negative control was media alone, the positive control was the ----------. The reduction of ---------- was monitored using ---------- assays on ----------. The assay was done in ---------- (See Appendix IIV-16).

1. ---------- Column (----------)
   Load Buffer: ----------.
   Elution Buffer: ----------.

	New resin	Used resin (--- cycles)
Input Viral Load (Log $_{10}$ PFU)	-----	-----
Output Viral Load (Log $_{10}$ PFU)	-----	-----
Log $_{10}$ Reduction	-------	-------

2. ---------- Column
   Load Buffer: ----------
   Elution Buffer: ----------

	New resin	Used resin (--- cycles)
Input Viral Load (Log $_{10}$ PFU)	-----	-----
Output Viral Load (Log $_{10}$ PFU)	-----	-----
Log $_{10}$ Reduction	--------	--------

3. ----------
   Load Buffer: ----------
   Elution Buffer: ----------

	New resin	Used resin (-- cycles)
Input Viral Load (Log $_{10}$ PFU)	-----	-----
Output Viral Load (Log $_{10}$ PFU)	-----	-----
Log $_{10}$ Reduction	-------	-------

4. Cytotoxicity/Interference Assays

   ----------cells was used to assess cytotoxicity and interference. The negative control consisted of ----------, the positive control was the ---------- The minimum dilution for virus titers in the cytotoxicity and interference assays was ---- (---- and -----columns) and ------- (------- column). These data were taken into account when evaluating the viral clearance studies.

Comments: The cumulative reduction of -------would be:

	New	Used
---------- Column	----------	----------
---------- column	----------	----------
---------- column	----------	----------
Total reduction:	----------	

The studies used appropriate infectivity assay methods and controls. There is a high level of clearance to ensure reduction ---------- particles. The sponsor states that the mechanism of action of the ---------- column could be either ----------.

C. ----------

This is a ------- ----, non-enveloped, dsDNA virus of the ---------- family. The negative control was ----------, the positive control was ----------. The reduction of ---------- was monitored using infectivity assays, in ----------. See Appendix IIV-16, vol 23.

1. ---------- Column
   Load Buffer: ----------.
   Elution Buffer: ----------

	New resin	Used resin (--- cycles)
Input Viral Load (Log $_{10}$ PFU)	------	------
Output Viral Load (Log $_{10}$ PFU)	------	------
Log $_{10}$ Reduction	------	------

2. ---------- column (----------)
   Load Buffer: ----------
   Elution Buffer: ----------

	New resin	Used resin (-- cycles)
Input Viral Load (Log $_{10}$ PFU)	------	------
Output Viral Load (Log $_{10}$ PFU)	------	------
Log $_{10}$ Reduction	------	------

3. ---------- column
   Load Buffer: ----------.
   Elution Buffer: ----------

	New resin	Used resin (--- cycles)
Input Viral Load (Log $_{10}$ PFU)	------	------
Output Viral Load (Log $_{10}$ PFU)	------	------
Log $_{10}$ Reduction	------	------

4. Cytotoxicity/Interference

   ---------- infectivity of -------- cells was used to assess cytotoxicity/interference. The negative control consisted of ----------, the positive control was the same ----------. No cytotoxicity or interference were found at a ------- dilution of the ------- column buffers. These data were taken into account when evaluating the viral clearance studies.

Comments: The cumulative log 10 reduction of ---------- would be:

	New	Used
------Column	----------	----------
------ column	----------	----------
----- column	----------	----------

Total reduction: ----------

The studies used appropriate infectivity assay methods and controls. There is --------- digit clearance for reduction of human ---------- particles. The sponsor states that the mechanism of action for ---------- removal by the ----------sepharose columns was due to ----------.

D. ----------

This is a non-enveloped ssRNA virus, approx. ---- nm, and part of the ---------- family. The negative control was ----------, the positive control was ---------- ----------. The reduction of --------- was monitored using infectivity assays, in ----------. See Appendix IIV-16.

1. ---------- Column
   Load Buffer: ----------.
   Elution Buffer: ----------.

	New resin	Used resin (--- cycles)
Input Viral Load (Log $_{10}$ PFU)	----	----
Output Viral Load (Log $_{10}$ PFU)	----	----
Log $_{10}$ Reduction	----------	---------

2. ---------- column
   Load Buffer: ----------.
   Elution Buffer: ----------.

	New resin	Used resin (-- cycles)
Input Viral Load (Log $_{10}$ PFU)	----	----
Output Viral Load (Log $_{10}$ PFU)	----	----
Log $_{10}$ Reduction	-------	-------

3. ---------- column

Load Buffer: ----------

Elution Buffer: ----------

	New resin	Used resin (-- cycles)
Input Viral Load (Log $_{10}$ PFU)	-------	-------
Output Viral Load (Log $_{10}$ PFU)	-------	-------
Log $_{10}$ Reduction	-------	-------

4. Cytotoxicity/Interference Assays

---------- infectivity in --------- was used to assess cytotoxicity/interference. The negative control was ----------, the positive control was ---------- ---------- ----------. Cytotoxicity and interference were found in some undiluted buffers, not in others. These data were taken into account when evaluating the viral clearance studies.

Comments: The reduction values from the ------- column are negligible. The cumulative Log $_{10}$ reduction of would be:

	New	Used
---- column	----------	----------
--- column	----------	----------

Total reduction: ----------

The studies used appropriate methods and controls in the infectivity assays. ---------- ---------- ---------- ---------- ---------- However, the in vitro broad screen assay for adventitious agents (which uses ----------) should detect small non-enveloped viruses such as ----------, though the virus would not be eliminated. The sponsor stated that the mechanism of clearance was ----------.

E. Summary of Virus Clearance:

Both new and used column resins were tested. The studies were done at ---------- ---------- The sponsor used 4 model viruses covering different physico-chemical properties (RNA/DNA, enveloped/non-enveloped, small/large size). ---------- ---------- were used. But, viruses with very high physico-chemical resistance were not tested (i.e. the ---------- families). These should have been included.

The indicator cell lines and infectivity assays were appropriate for the viruses tested and were done in replicates. Cytotoxicity and interference assays were done. Also, the

sponsor stated the mechanism of clearance (----------) for some of the column steps (Vol. 23, pages 14-15).

Log $_{10}$ reduction values were in the ---------- digits for the viruses tested, with the exception of ----------. In the initial clearance study, the reduction value for ------ was -----, in the second study this was reduced to ---- (for both new and used resin). Small, nonenveloped viruses (i.e. -----) will not be sufficiently cleared by the chromatography columns if they enter the manufacturing process. ----------.

CHO cells are known to contain endogenous rodent retrovirus. The ----------digit clearance achieved for enveloped viruses seems sufficient, however, this cannot be known until the sponsor quantifies the starting retroviral load in the lot.

The BLA provided data quantifying the retroviral-like particle load in three fermentation batches representative of ---------- processing used for production of Fabrazyme. This was obtained for both the cell culture harvest and the ------- (first column) load (clarified harvest). The sponsor stated that there could be --- Log $_{10}$ retrovirus-like particles per dose. Given the clearance of ----- Log $_{10}$, this means that ----- Log $_{10}$ clearance would remain, which in turn would result in there being one particle per --- doses. The sponsor did not evaluate based on the worst case scenario. They incorrectly used ---------- of the -------- load (---------- particles/ml). Using the worst case number of -------- particles/ml for the --------- load, the particles per dose would be ------ Log $_{10}$. So, in turn, there could be approximately one particle per --- doses.

F. Column Cleaning/Solution Inactivation Studies

1. ------ Column Inactivation Studies with ----------:

(See Table IIV-24) The purification process of r-hαGAL contains a ---------- step incorporated into the ------ column wash buffer.

Virus	Titer T=0min	Log$_{10}$ Reduction T=10min	T=30min	T=60min
----------	----	------	------	------
----------	----	------	------	------
-----	----	------	------	------
-----	----	------	------	------

Infectivity assays on either ---------- cells (----------) were used. For the ---------- column, over -- logs of the enveloped viruses were inactivated within --- minutes. This wash with ---------- would help to reduce carryover of enveloped viruses to the next batch, and reduce any potential virus in downstream processing.

2. Column Cleaning:

Solution 1 is used to clean the ---------- column and consists of ---------- Solution 2 is used to clean and sanitize the -- remaining columns and consists ----------. Each solution was

incubated with virus, ----------, diluted, and plated onto indicator cells. See Table IIV-32.

During full-scale manufacture, the columns are exposed to the cleaning solutions as follows:

Column	Solution	-----. Contact Time
----------	----------, ---------- ----------	----------
----------	---------- ----------	----------
----------	---------- ----------	----------
----------	---------- ----------	----------

From Table IIV-32:

Virus	Sample	-------	Log $_{10}$ Reduction -------	-------
---------- ----------	Solution 1	--------	--------	--------
	Solution 2	----	--------	--------
----------	Solution 1	--------	--------	--------
	Solution 2	----	----	----
------	Solution 1	--------	--------	--------
	Solution 2	--------	--------	--------
---------	Solution 1	--------	--------	--------
	Solution 2	----	----	----

---------- (Spiking Virus Titer = ---------- PFU/ml)
---------- (Spiking Virus Titer = ---------- PFU/ml)
---------- (Spiking Virus Titer = ---------- PFU/ml)
---------- (Spiking Virus Titer = ---------- FFU/ml)

Comments: Both solution 1 and solution 2 reduced all four viruses tested by at least --- logs within ----------. There was little change with longer exposure times except for ----------, which was reduced by -- logs within -------- with solution 2. Potential virus remaining on the columns should be inactivated by at least 5 logs, to reduce carryover to the next process batch.

The column cleaning/inactivation studies were done ---------- ---------- Viral and protein buildup on a column may potentially change the chemical resistance of a virus. The sponsor should determine whether the study performed is relevant to the cleaning in the actual manufacturing process.

G. Fabrazyme Obtained From Scaled-down vs. Full-scale manufacture:

(See Table IIV-18) ---------- eluate) and ---------- (all other eluates) were used to determine ---------- of the elution fractions of the various chromatography steps. The percent -------- obtained for the scaled-down process met specifications set for full-scale manufacturing runs. ----------, as determined by an ---------- assay and by ----------, met specifications set for full-scale manufacturing runs. Also the ---------- profile for the scaled-down process, as determined by --------, met specifications set for full-scale manufacturing runs.

Thus the scaled-down process was representative of full-scale manufacturing.

III. Validation Data for Detection of Adventitious Agents

A. Review of test methods for the MCB, WCB, -----, virus clearance:

Mycoplasma and sterility assays
---------- for retroviruses
---------- for adventitious agents
Infectivity assays (----------, and model viruses)
---------- test
---------- murine retrovirus assays
Isoenzyme analysis

The test methods used appropriate controls and validated procedures for characterization of the MCB, WCB and ---- and also for virus clearance. Tests conformed to standard methods and to requirements for valid assays. The assays were done in replicates, showing reproducibility.

IV. Action Items

A. Please provide results from the validation study performed to determine the sensitivity of the ---------- analysis for the detection of contaminating species in the cell banks and in ---------- cells.
B. Please identify and quantify the ---------- obtained from the cell banks and ---- cells.
C. Studies on viral clearance/inactivation during cleaning of resins were not performed in the presence of ----------. Please provide data that support the relevance of these studies in providing assurance that in case of a viral buildup on a column, the cleaning procedures would effectively remove or inactivate viruses.
D. In a worst case scenario analysis, based upon the total retrovirus-like particle load and the overall viral clearance of a model retrovirus (----------), the Fabrazyme manufacturing process could contain one retrovirus-like particle per ---- doses of Fabrazyme. This level of viral clearance is not considered sufficient. Please reevaluate the Fabrazyme manufacturing process for its capacity to remove retrovirus particles in order to demonstrate that the purification process is able to remove substantially more virus particles than is estimated to be present in a single dose

equivalent of starting material. Include with your reevaluation, a detailed description of how the estimated number of virus particles per dose equivalence is calculated.

E. Preparation of a new Working Cell Bank from the existing Master Cell Bank will use ----------